Research Article

Quantification of Vitamin C Content in *Rosa brunonii* Lindl Rosehips: A Potential Vitamin Supplement

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Abstract | Vitamin C (L-ascorbic acid) is as vital to flora as it is to animals. It is modulating the regeneration of antioxidants, hormone biosynthesis, and photosynthesis. This study includes the collection of *Rosa brunonii* rosehips from various locations in Murree Hills and the quantification of vitamin C in these samples. The average weight of rosehips varied from 4.54 to 7.92 g. Rosehips collected from Lower Topa (LT) showed the highest weight with a mean value of 7.92 g, followed by rosehips collected from Danoi (DA) (7.25 g). The average vitamin C content varied from 918 to 2985 mg/100 g. Whereas the total soluble solids (TSS), and total dry weight (TDW) varied from 31 to 34% and 34 to 40%, respectively. Rosehips collected from Bhurban (BH) showed the highest vitamin C content with 2985 mg/100 g along with 36% TSS and 40% TDW, followed by rosehips from Ghora Gali (GG) that had 2535 mg/100 g vitamin C content along with 35% TSS and 39% TDW. The difference in vitamin C content could be attributed to climate, including temperature and rainfall patterns, which directly influence plant growth and metabolism. Rosehips are used in the production of a variety of sweets and beverages, including syrup, marmalade, jellies, and jams. Therefore, high vitamin C content as well as TDW and TSS characteristics for rosehips are desired and preferable.

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Introduction

Vitamin C is an antioxidant that is soluble in water and primarily functions in a protective capacity. The antioxidant properties of vitamin C enable it to effectively scavenge free radicals, thereby protecting lipids, proteins, and DNA from oxidative damage (EFSA Panel on Dietetic Products *et al*., 2017). It also helps plants fight against oxidative stress caused by biotic or abiotic stressors due to its antioxidant capabilities and excellent redox regeneration mechanism (Gest *et al*., 2013). The significance of vitamin C in being able to resist various stressors is emphasized by the upregulation of biosynthetic and recycling enzymes in response to unfavorable environmental circumstances (Gallie, 2013; Höller *et al*., 2015). It reduces iron in the active site of several dioxygenases to Fe2+ and post-translationally modifies procollagen to produce collagen. It is a cofactor for several enzymes that are responsible

for the hydroxylation of proline and lysine, which is essential for the formation of the stable structure of collagen (Mandl *et al*., 2009; Pekkala *et al*., 2003; Peterkofsky, 1991). It helps hydroxylase synthesize carnitine and norepinephrine (Dunn *et al*., 1984; Rebouche, 1991). Vitamin C also plays a significant role in various hydroxylation reactions that are dependent on cytochrome P450. These reactions include the conversion of cholesterol into bile acids, the breakdown of exogenous substances like drugs and pollutants, and the production of steroid hormones (Kobayashi *et al*., 2014).

Vitamin C is categorized as a vitamin solely for selected vertebrate species, like Homo sapiens, due to their inability to endogenously produce it. The human incapacity to produce vitamin C is attributed to the functional deficiency of the gene encoding l-gulonolactone oxidase, which is the final enzyme in the animal biosynthesis of this particular vitamin (Linster and Van Schaftingen, 2007). Ascorbate plays a crucial role in the biosynthesis of collagen and carnitine in animals, which are essential components of blood vessels, tendons, ligaments, scar tissue, and skin (Levine, 1986; Vissers *et al*., 2020). The significance of vitamin C lies in its crucial role in the maintenance and repair of teeth, bones, and cartilage, as well as its involvement in the process of wound healing. In recent years, there has been significant interest in enhancing the nutritional value of plants by increasing their vitamin C content, given that plant-based foods are the primary source of vitamin C in human diets (Chen *et al*., 2003; Hancock and Viola, 2005; Naqvi *et al*., 2009). According to some research, vitamin C's antioxidant properties make it effective against cancer, cardiovascular disease, and atherosclerosis (Harris, 2013).

Rosa brunonii pertains to the botanical family Rosaceae. This particular plant species holds significant importance in the field of medicine. It is a spindly climbing shrub that can reach a height of 5-6 meters. Its scented flowers, which typically bloom from April to June, are characterized by white petals. *R. brunonii* is found growing as a wild shrub in various regions of Pakistan, including Gilgit, Kashmir, Poonch, Hazara, Swat, Chitral, Kurram, Murree, and Balouchistan (Ishaque *et al*., 2021). It has been utilized in traditional medicine to address a range of health issues. The bark infusions are employed as a means of purifying the blood, while the leaf extract is applied externally to promote wound healing when mixed with pure water. Additionally, the tea of the flowers is utilized to alleviate diarrhea and constipation, and the flower extract is employed to address skin and eye ailments. The local inhabitants of the Galliyat region in Pakistan have also been known to use the fruit of *R. brunonii* to treat skin diseases (Amjad *et al*., 2017; Bano *et al*., 2014; Ishaque *et al*., 2017; Khan *et al*., 2015). This paper will examine and evaluate the content of vitamin C in *Rosa brunonii* rosehips from various Murree locations focusing its potential as vitamin supplement. Rose hips are a nutritious and versatile addition to the diet. They have been consumed for centuries due to their health benefits and culinary uses. Quantifying vitamin C in rose hips for vitamin supplements is crucial for ensuring accuracy, product consistency, health benefits, and consumer confidence. It helps both manufacturers and consumers make informed decisions about the use of rose hip supplements in their diets.

Materials and Methods

Materials and reagents

Plant material (rosehips of *Rosa brunonii*) were collected from five localities of Murree hills i.e., Ghora Gali (GG), Danoi (DA), Kotli Sattian (KS) Bhurban (BH), and Lower Topa (LT) at an elevation ranging from 1000 to 2000 meters. Chemical reagents including ammonium molybdate, oxalic acid, Sulphuric acid, EDTA, metaphosphoric acid, acetic acid, and L-ascorbic acid were purchased from Sigma Aldrich and used as received. All the solvents used in this study were HPLC grade or distilled before use.

Sampling and physical examination of rosehips

Three sets of twenty rosehips prepared by randomly picking up from each sample collected from five different localities of Muree Hills. Each set of twenty rosehips were assessed to measure average fruit weight, flesh ratio, fruit length, and fruit width of rosehips.

Vitamin C extraction

To extract vitamin C from rosehips, first an oxalic acid-EDTA solution was prepared by dissolving 6.3 g of oxalic acid and 744 mg of EDTA in 1000 mL distilled water (Bajaj and Kaur, 1981). Subsequently, 10 g of each sample were blended in 100 mL oxalic acid-EDTA solution to prepare homogenized suspension through electric blender. The resultant suspension was filtered to remove the rosehips residue and large solid

particles. The filtrate was collected and then centrifuge at 3000 rmp to remove any micron level particles. The supernatant was transferred to amber reagent bottle, properly labeled, and stored for farther analysis.

Devolvement of calibration curve

A stock solution of vitamin C with a concentration of 0.1% (m/V) was prepared through the dissolution of 100 mg of L-ascorbic acid in a 100 mL solution of oxalic acid-EDTA. To construct the calibration curve, discrete volumes of 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mL were transferred into individual volumetric flasks that were pre-filled with a 50 mL solution of oxalic acid-EDTA. To each flask, an additional 5 mL of metaphosphoric acid-acetic acid solution was added, followed by 10 mL of a 10% (v/v) sulfuric acid solution, and finally 20 mL of ammonium molybdate solution. The solution obtained was subjected to dilution using distilled water until a final volume of 250 mL was achieved. Subsequently, the absorbance of the solution was measured at 760 nm using spectrophotometric techniques. The solution of oxalic acid and EDTA was prepared through the dissolution of 6.3 g and 744 g of oxalic acid and EDTA in 1000 mL of distilled water, respectively. A solution of metaphosphoric acid and acetic acid was produced through the dissolution of 15 g of metaphosphoric acid in 40 mL of acetic acid, followed by the addition of distilled water to achieve a final volume of 500 mL. The preparation of an ammonium molybdate solution involved the dissolution of 5 g of ammonium molybdate in 100 mL of distilled water.

Quantification of vitamin C

To quantify the vitamin C in rosehips samples, all the vitamin C extraction samples stored in amber reagent bottle were shake well and then 50 mL extraction of each sample was transferred into individual volumetric flasks. To each flask, an additional 5 mL of metaphosphoric acid-acetic acid solution was added, followed by 10 mL of a 10% (v/v) sulfuric acid solution, and finally 20 mL of ammonium molybdate solution. The solution obtained was subjected to dilution using distilled water until a final volume of 250 mL was achieved. Subsequently, the absorbance of the solution was measured at 760 nm using spectrophotometric technique.

Results and Discussion

Physical examination of rosehips

The physical properties of the rosehips collected from five different localities are shown in Table 1. The weights and dimensions of representative rosehips were recorded as minimum (Min), maximum (Max), and mean values (Average). The mean weights of the rosehips exhibited a range between 4.54 and 7.92 g. The results indicate that LT exhibited the greatest mean weight of 7.92 g, followed by DA (7.25 g). BH and KS had almost similar average weight of rosehips whereas GG had the lowest wight among all samples. Average rosehip flesh ratio varied from 62.35% to 77.13%. The weight of a rosehip is an important factor in determining its nutritional value, as larger rosehips generally contain more nutrients. The flesh ratio, which refers to the ratio of pulp to seeds within the rosehip, is also important, as the pulp contains more nutrients than the seeds. Generally, rosehips with a higher flesh ratio are considered to be of higher nutritional value. Average fruit width was ranging from 6.45 to 9.65 mm. Average fruit length was found in the range of 12.15 to 16.93 mm. The length and width of rosehips can also affect their nutritional value. Larger rosehips may contain more flesh and seeds, which can contribute to their overall nutritional content. However, it is important to note that other factors, such as the variety of rosehip and the conditions in which it was grown, can also impact its nutritional content (Ercişli and Eşitken, 2004; Lee and Kader, 2000).

Table 1: *Rosehip weight, rosehip flesh ratio, rosehip length, and rosehip width of Rosa Brunonii collected from five different localities of Muree Hills i.e., Ghora Gali (GG), Danoi (DA), Kotli Sattian (KS) Bhurban (BH), and Lower Topa (LT).*

Samples	Rosehip weight (g)			Rosehip flesh ratio (%)			Rosehip width (mm)			Rosehip length (mm)		
	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average
GG	4.23	4.64	4.54 ±0.15	56.34	65.27	62.35 ±0.25	5.78	7.15	6.45 ±0.23	9.67	14.45	12.15 ±0.45
DA	5.95	7.84	7.25 ±0.12	67.58	78.94	75.18 ±0.28	7.74	9.12	8.63 ±0.25	11.86	18.24	15.84 ±0.23
KS	5.86	7.32	6.18 ±0.17	65.73	68.25	67.36 ±0.35	6.45	8.38	7.35 ±0.15	10.38	16.85	14.15 ±0.16
BH	5.75	7.96	6.35 ±0.15	66.86	71.49	69.75 ±0.18	6.36	8.96	7.57 ±0.25	12.72	17.18	14.75 ±0.43
LT	6.67	8.32	7.92 ±0.14	72.92	84.35	77.13 ±0.23	8.58	10.45	9.65 ±0.32	13.65	19.63	16.93 ±0.45

Links Researchers

Devolvement of calibration curve

Several techniques have already been reported for determining vitamin C. The spectrophotometric or calorimetric methods are commonly employed, depending on the reactions with phenylhydrazinium chloride, dimethoxyquinone, 4-nitrobenxenediazonium fluoroborate, 2, 4-dinitrophenylhydrazine, diazotized 4-methoxy-2-nitroaniline, and 2, 6-dichloroindophenol (Besada, 1987; Kelly and Latzko, 1980; Khadka and Pathak, 2023; Soliman, 1979). The molybdophosphate complex has also been identified as a means of determining vitamin C content (Vishnikin *et al.*, 2011). The initial trials indicated that the response depends on the proportion of sulfuric acid to vitamin C. The stability of the blue color generated persists for approximately 20 hours at room temperature. The spectral absorption peak occurs at a wavelength of 760 nm, and the linear relationship between the concentration of the solution and its absorbance, defined by Beer's law, is valid within the concentration range of 2–25 µg/mL (Figure 1).

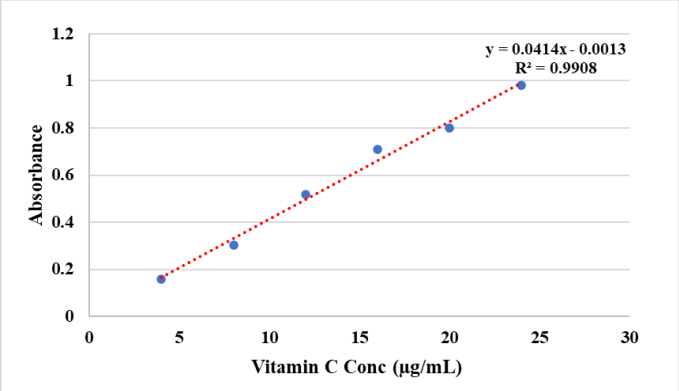

Figure 1: *Calibration curve for quantification of vitamin C spectrophotometrically at 760 nm.*

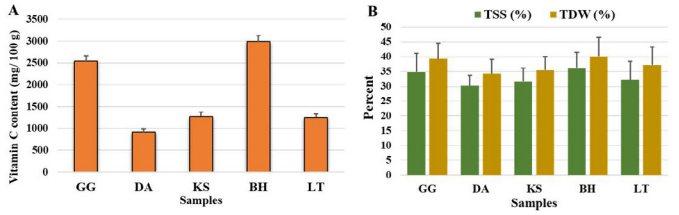

Figure 2: *Vitamin C content (VTC), TSS, and TDW of rosehips collected from five different localities of Muree Hills i.e., Ghora Gali (GG), Danoi (DA), Kotli Sattian (KS) Bhurban (BH), and Lower Topa (LT).*

Quantification of vitamin C

Vitamin C content, total soluble solids (TSS), and total dry weight (TDW) of the rosehips collected from five different localities are shown in Figure 2. The average vitamin C content varied from 918 to 2985 mg/100 g. Whereas the TSS and TDW varied from 31 to 34% and 34 to 40%, respectively. BH had the highest vitamin C content with 2985 mg/100 g along with 36% TSS and 40% TDW, followed by GG, which had 2535 mg/100 g vitamin C content along with 35% TSS and 39% TDW. KS and LT had almost similar vitamin C content, whereas DA had the lowest weight among all samples. Typically, fresh consumption of rosehip fruits is not a common practice. The fruits undergo processing procedures to yield various consumable products such as soft drinks, syrup, marmalade, jellies, and jams (Ahmad and Anwar, 2016). Thus, the desirable and significant properties of rosehips include higher levels of vitamin C content, TSS, and TDW.

There are several factors affecting the content of vitamin C in wild rosehips. The stage of maturity of the rosehips affects the vitamin C content. Generally, rosehips that are fully ripe contain more vitamin C than those that are not yet fully mature (Medveckienė *et al.*, 2021). The harvesting time can also affect the vitamin C content (Kallio *et al.*, 2002). Rosehips that are harvested in the late fall or early winter generally have a higher vitamin C content than those harvested earlier in the season. The processing method used to prepare the rosehips affects the vitamin C content. Heat, light, and air all cause vitamin C to break down, so processing methods that minimize exposure to these factors help preserve the vitamin C content in rosehips (Santos and Silva, 2008). The storage conditions of the rosehips affected the vitamin C content. Storing rosehips in a cool, dark, and dry place also helps preserve the vitamin C content (Franke *et al.*, 2004).

Conclusions and Recommendations

In summary, this study was conducted to quantify the vitamin C content in rosehips collected from various locations in the Murree Hills. The vitamin C content of the samples ranged from 918 to 2985 mg/100 g, with BH and GG having the highest vitamin C content. The vitamin C content of rosehips is affected by a variety of factors, so it's important to take these factors into consideration when harvesting, processing, and storing the rosehips. The results concluded that rosehip from Rosa Brunonii is a rich source of vitamin C, and samples collected from various locations had a significant content of vitamin. Rosehips from these locations could be used

as a potential vitamin supplement in producing syrup, marmalade, jellies, and jams.

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Novelty Statement

Rose hips are a nutritious and versatile addition to the diet. Quantifying vitamin C in rose hips for vitamin supplements is crucial for ensuring accuracy, product consistency, health benefits, and consumer confidence.

Author's Contribution

S. Saifullah: Conceptualization, Methodology, Data curation, Data interpretation, Data visualization, and writing first draft and writing revised drafts. M. Ilyas: Methodology, Data curation, and writing first draft. B. Ullah: Methodology, Data curation, and writing first draft. S. Z. Satti: Conceptualization, Supervision, Plant material collection, Reviewing and Editing original draft and revision.

Conflict of interest

The authors have declared no conflict of interest.

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